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Rate and Severity of HIV-Associated Dementia (HAD): Correlations with Gp41 and iNOS

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Abstract

Background: Fifteen to thirty percent of AIDS patients develop some type of neurologic disorder during the course of their illness and the vast majority of these neurologic disorders will be HIV-associated dementia (HAD). These patients can exhibit varying degrees of severity and rates of progression of HAD. Neuropathologic variables that are associated with the rate of progression of HAD are not known.

Materials and Methods: Tissue was collected at autopsy from the Johns Hopkins University HIV Neurology Program. Seventy-one AIDS patients of this prospectively characterized population were followed until death to obtain information on dementia severity and the rate of neurological progression. Immunoblot analysis of immunological nitric oxide synthase (iNOS), HAM56, gp41, p24, gp120, and β-tubulin was performed and the levels of iNOS, HAM56, gp41, and p24 were normalized to β-tubulin and analyzed for significance by means of the Kruskal-Wallis test for multiple groups.

Results: We have identified unique groups within this spectrum and designated them slow, moderate, and rapid progressors. Slow and moderate progressors' neurological progression occurs over a course of months to years, whereas the rapid progressors' disease shows rapid increases in severity over weeks to months. In the present study we demonstrate that the severity and rate of progression of HAD correlates significantly with levels of the HIV-1 coat protein, gp41, iNOS, and HAM56, a marker of microglial/macrophage activation.

Conclusion: The severity and rate of progression of HAD correlates with indices of immune activation as well as levels of iNOS and gp41. There appears to be a threshold effect in which high levels of gp41, iNOS, and immune activation are particularly associated with severe (Memorial Sloan-Kettering score 3 to 4) and rapidly progressive HAD.

Introduction

Fifteen to thirty percent of AIDS patients have neurologic disorders such as HIV-associated dementia (HAD), minor cognitive/motor disorder, HIV-associated myelopathy, and HIV-associated sensory neuropathy (1). These are among the most common causes of neurologic disease in young adults in the United States today (1). HAD has an overall prevalence among HIV-infected children and adults of 15 to 20%, and an annual incidence in patients with AIDS of 5 to 10% (1). Twenty to thirty percent of AIDS patients will develop HAD during the course of their illness (2–4) and higher incidences have been reported in intravenous drug users and women (1).

Human immunodeficiency virus type-1

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(HIV-1) preferentially infects $CD4^+$ cells of the peripheral immune system and it also targets $CD4^+$ immune cells of the central nervous system, primarily microglia and perivascular macrophages, early in the course of HIV-1 infection (5,6). Despite the occasional infection of astrocytes and the rare infection of neurons (7–10), there is cortical neuronal and synaptic loss and dendritic simplification (11–13). Therefore, indirect mechanisms are most likely to be involved in the pathogenesis of HAD (14,15). Neuropathologic studies indicate that HAD may be related to levels of unintegrated viral DNA, multinucleated giant cells, positive immunostaining for gp41 and p24 (16), and immunostaining for a unique subset of apoptotic-inducing CD14/CD16-positive and CD14/CD69-positive monocytes (17). Psychomotor slowing as well as increased levels of cerebrospinal fluid (CSF) β2-microglobulin and neopterin and decreased CD4 counts (18) carry an increased risk of progression to dementia (19).

The severity of HAD may also correlate with the number of HIV-1-infected macrophages (5,20–22), and CSF RNA viral burden (23). Recently we showed that levels of gp41 as ascertained by Western blot analysis correlate with the severity of HAD and the levels of immunological nitric oxide synthase (iNOS) (24). Gp41 is also increased in pediatric patients with progressive neurologic disease (25). In SIV-infected macaques, neurologic disease correlates with gp41 expression, but not with viral load as detected by viral message (J. Clement and M. C. Zink, personal communication), paralleling observations made in HAD. Expression of mRNA for iNOS, tumor necrosis factor-α (TNF-α), and macrophage inflammatory protein α (MIP-1α) and β (MIP-1β) is in close proximity to gp41-positive cells in HAD patients (26). MIP-1α and MIP-1β are potent chemoattractants for both monocytes and specific subpopulations of lymphocytes. In rodent neuronal/glial and human neuronal aggregate cultures, physiologic subnanomolar concentrations of gp41 induce a cascade of events that is neurotoxic by inducing iNOS- and nitric oxide (NO)–dependent neurotoxicity (24; L. Pulliam, personal communication). Thus, in addition to other viral proteins and host factors, gp41 may play a key role in the pathogenesis of HAD.

The rate of progression of HIV infection to AIDS is influenced by factors including older age (27,28), lower $CD4^+$ cell count (27), decreased hemoglobin, reduced platelet count, and elevated IgA levels and thrush (29); however, disease progression is not associated with race, sex, or risk factor for HIV infection (30). Variations in viral genotype, production by infected macrophages, and subsequent toxin production may contribute to the variability in neuropathology (31) and likewise the variability in neurological progression of HAD. Many studies describing characteristics of HAD looked primarily at cases of rapid progression over a few months, with a mean survival of 3 to 6 months (32–35); few have given attention to differences among variable rates of progression of HAD (36) in relation to neuropathologic correlates. We report here that the severity and rate of progression of HAD correlate with levels of gp41 and iNOS.

Patients and Methods

Study Subjects

Using the American Academy of Neurology (AAN) criteria (37) (equivalent criteria for cases before 1991), the Johns Hopkins University HIV Neurology Program diagnosed 329 adult patients with HAD between 1984 and 1994. Seventy-one of these patients were followed until death to obtain information on dementia severity and rate of neurological progression. All of these patients received neurological or neuropsychological examinations within 3 months of death, had a follow-up of >1 month, and had initial Memorial Sloan-Kettering (MSK) scores (38) of <3. None of these patients had clinical or pathological evidence of pre-existing or concurrent central nervous system (CNS) opportunistic infections, confounding illicit drug use, or pre-existing psychiatric disorders.

Rate of neurological progression was ascertained according to the change in the MSK dementia severity score (38) at initial diagnosis and at death. The criteria for classification of progression rates are described by Bouwman and colleagues (36) in detail and for purposes of brevity are not repeated here. On the basis of these calculated rates of dementia progression, the group was divided into tertiles and compared according to demographic, clinical, and laboratory characteristics. For this study, 11 of these patients were randomly selected, along with HIV-positive, nondemented individuals, for protein analysis of postmortem CNS tissue. The nondemented, slow, moderate, and rapid progression groups each had eight, three, four, and four individuals, respectively. For purposes of statistical power and further assessment of neuropathologic variables associated with severity of dementia, we also combined these immunoblot results

with previous findings (24) and examined potential correlations with severity of dementia at time of death. For this analysis, we had a total of 26 samples from individuals with MSK scores at time of death ranging from 0 to 4.

Demographic and Clinical Data

Baseline demographic information included age, survival from time of HAD diagnosis, CD4 count near time of death, and postmortem interval (time from death to fixation or freezing of CNS tissue). Other demographic and clinical data collected included race, sex, risk factor for HIV infection (homo- or bisexual, heterosexual, injection drug use, blood transfusion, or unknown), antiretroviral use, AIDS-defining illnesses, radiological results, presenting signs and symptoms, clinical staging of HIV disease, results from neuropsychological testing including Mini Mental State Exam scores (39) or HIV-dementia scale (40), as well as a complete neurological history. These other data are reported in detail elsewhere (36) and are not repeated here.

Immunoblot Analysis

Tissue was collected at autopsy from this prospectively characterized population of AIDS patients, was rapidly frozen in isopentane, and stored at −70°C. Cortical specimens were obtained from the mid-frontal gyrus from all patients. In all patients, the presence of CNS opportunistic infections or lymphoma was excluded by computed tomography (CT) or magnetic resonance imaging (MRI) and CSF analysis, as well as by postmortem histopathologic evaluation of brain tissue sections. HIV-1-seronegative control specimens were obtained from patients without CNS lesions and have been previously reported (24). The causes of death in control patients included myocardial infarction, trauma, cirrhosis of the liver, atherosclerosis, and widespread cytomegalovirus infection.

Immunoblot analysis of iNOS, HAM56, gp41, p24, gp120, and β-tubulin was performed as described previously (24). Briefly, tissues were homogenized in ice-cold 50 mM Hepes (pH 7.4) with 1 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamide, leupeptin (10 μg/ml), pepstatin A (10 μg/ml), aprotinin (1 μg/ml), and 1 mM EDTA, and centrifuged at 100,000 × *g* for 60 min. Five to sixteen percent gradient polyacrylamide gel electrophoresis (PAGE) was used to separate proteins. After electrophoresis, proteins were electroblotted onto nitrocellulose and incubated with anti-macNOS antibody (1:500, Transduction Laboratories, Lexington, KY), anti-macrophage "HAM56" antibody (1:1000, Enzo Diagnostics, Farmingdale, NY), anti-gp41 antibody (1:250, #1577, Intracel, Cambridge, MA), anti-p24 (HIV-1) antibody (1:1000, Intracel), anti-gp120 (HIV-1) antibody (1:250, Intracel), and anti-β-tubulin antibody (1:10000, Sigma Immuno Chemicals, St. Louis, MO), respectively. The anti-gp41 antibody is epitope mapped to gp41 amino acids 733–750 and recognizes only one protein that runs at 41 kD only in HIV-infected patients (24). We have never detected immunoreactivity by Western blot analysis in HIV-seronegative controls. Furthermore, the gp41 epitope (aa 733–750) does not have any significant homology to any currently known human proteins as determined by both BLAST and BLITZ protein sequence searches. The anti-gp120 antibody recognizes recombinant gp120 and native gp120 from extracts of HIV-1-infected cells in Western blots (24). For gp41 protein analysis, equivalent amounts of protein lysate prepared from each of the pellet fractions after a 60-min 100,000 × *g* spin were resolved using 4–20% gradient PAGE. Sodium dodecyl sulphate (0.1%) was added to the Tris-glycine buffer and Tween 20 (0.1%) was added to the phosphate-buffered saline (PBS) for rinsing steps. Immunoblots were developed by enhanced chemoluminescence (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Bands were scanned (Molecular Dynamics, Sunnyvale, CA) and relative ratios of the intensity of the bands to the β-tubulin band were calculated.

Statistical and Correlation Data

The levels of iNOS, HAM56, gp41, and p24 normalized to β-tubulin were analyzed for significance by means of the Kruskal-Wallis test for multiple groups. Data are reported here as means ± SEM. Fisher's least-significance difference posthoc test was used for each protein and comparison of significance was made between each neurological progression group and MSK scores. Protein level versus rate of neurological progression, and protein levels versus MSK score scattergram plots were generated by a bivariate regression analysis program. Nonparametric Spearman rank correlation tests were used to compare protein levels with rate of neurological progression and with MSK score at time of death.

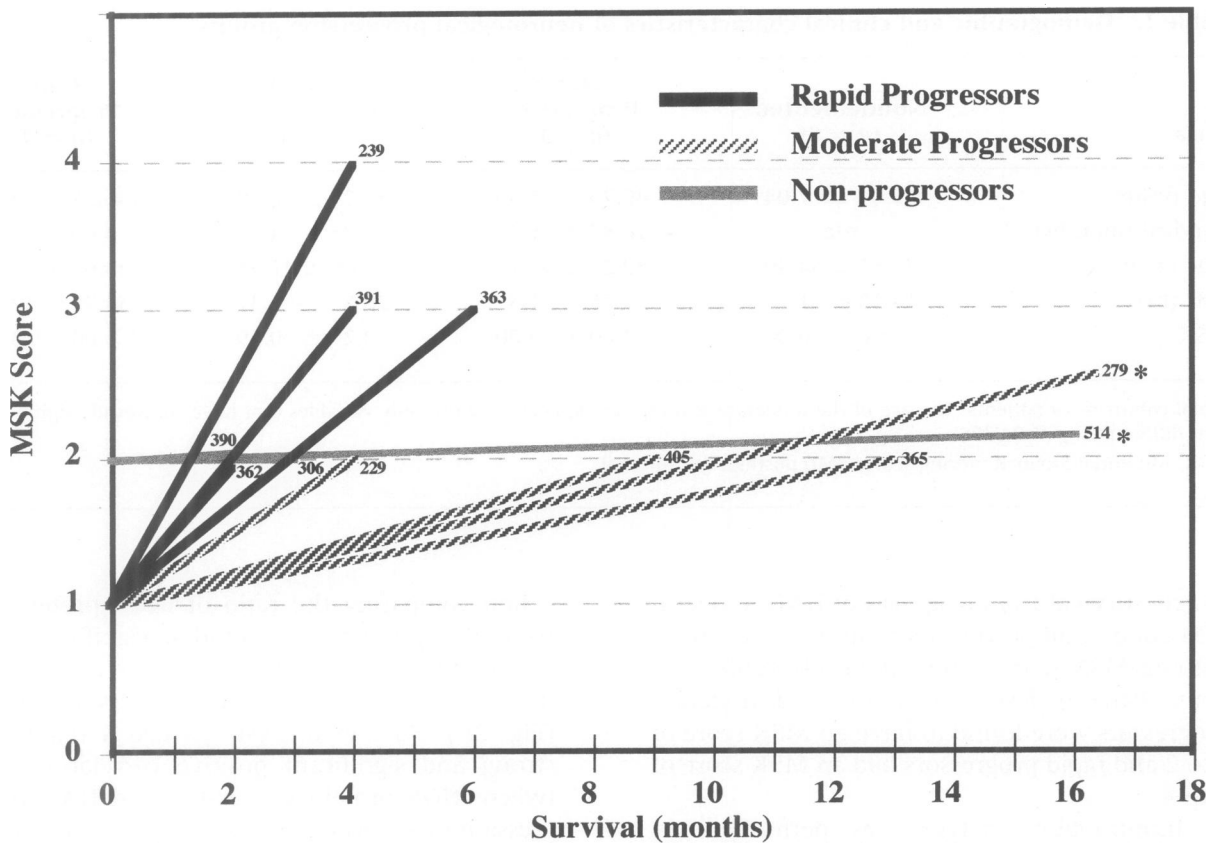

Fig. 1. Different rates of neurological progression among non-progressors (NP), moderate progressors (MP), and rapid progressors (RP). Seropositive nondemented cases are not shown since they have no survival value. The mean Memorial Sloan-Kettering (MSK) slope of the NP, MP, and RP groups was 0.000747 (±0.000606), 0.00461 (±0.00156), and 0.016842 (±0.003286), respectively. The median MSK slope of each group was 0.000557, 0.00349, and 0.01608, respectively (NP and MP, $p \leq 0.0064$; NP and RP, $p \leq 0.0109$; MP and RP, $p \leq 0.005$). *Survival for case 279 and 514 was 21 and 59.2 months, respectively.

Results

After selecting a group of patients from the original 71 patients, we divided the group into three on the basis of the rate of change in their MSK score from time of diagnosis to near or at the time of death. The mean MSK slope values for these groups were 0.000747 (±0.000606), 0.00461 (±0.00156), and 0.016842 (±0.003286), respectively (Fig. 1), and the median MSK slope values were 0.000557, 0.00349, and 0.01608, respectively. We designated these groups non-progressors, moderate progressors, and rapid progressors (non-progressors and moderate progressors, $p \leq 0.0064$; non-progressors and rapid progressors, $p \leq 0.0109$; moderate progressors and rapid progressors, $p \leq 0.005$). Some of the moderate and rapid progressors had slightly lower MSK scores than the non-progressors. In our analysis of HAD patients with different rates of progression we included a group of HIV-positive individuals who remained free of HAD during their lifetime. There was no significant difference among groups with regard to age at the time of death except between moderate progressors and rapid progressors (Table 1). These latter two groups had mean ages of 37.25 ± 0.99 and 46.25 ± 3.84, respectively ($p \leq 0.0396$). There were no significant differences with regard to length of survival from time of diagnosis of HAD to death among the groups. There were also no significant differences regarding CD4 counts and postmortem interval.

For purposes of statistical power and further assessment of variables associated with severity of dementia, we combined these immunoblot results with previous findings (24) and divided the combined results into three groups on the basis of MSK score at time of death (Table 2). These groups consisted of MSK scores of 0, 1 to 2, and 3 to 4, and showed no significant difference

Table 1. Demographic and clinical characteristics of neurological progression groups[a]

Data	Nondemented (n = 8)	Non-Progressors (n = 3)	Moderate Progressors (n = 4)	Rapid Progressors (n = 4)
Age (years)	45.00 ± 7.04	40.75 ± 5.50	37.25 ± 0.99	46.25 ± 3.84
Survival (months)	n/a	16.80 ± 28.27	11.75 ± 4.15	4.00 ± 0.94
CD4 count	76.67 ± 54.89	50.25 ± 55.17	77.00 ± 47.38	23.00 ± 13.47
PMI (hr)	14.38 ± 1.79	7.75 ± 1.5	20.5 ± 6.37	54.75 ± 52.12
MSK	0.00 ± 0.00	2.00 ± 0.00	2.25 ± 0.29	3.00 ± 0.47

[a]Total cohort = 19 patients. The age of the moderate and rapid progressors are the only variables that have statistically significant differences. $p \leq 0.0396$.

MSK, Memorial Sloan-Kettering score; PMI, postmortem interval.

in age, survival from diagnosis of HAD to death, CD4 count, and postmortem interval. The group with an MSK score of 0 were nondemented patients. Patients described as non- and moderate progressors were found to have an MSK score of 1 to 2 and rapid progressors had an MSK score of 3 to 4.

Immunoblot analysis was performed on samples from eight HIV-positive nondemented patient samples, three non-progressors, four moderate progressors, and four rapid progressors. High levels of iNOS protein expression were observed in all rapid progressor samples, in half of the moderate progressors, and at an extremely low level in one of the eight nondemented patients (Fig. 2A). To control for different amounts of protein loaded in each lane of the immunoblot, we also probed the same immunoblots for the ubiquitously expressed β-tubulin protein. When comparing the ratio of iNOS protein to β-tubulin protein, we found a significant increase in the amount of iNOS protein expression in the moderate and rapid progressor groups (Fig. 2B). Further analysis yielded a relatively strong and significant positive correlation between iNOS protein expression and HAD progression ($\rho = 0.611$, $p \leq 0.010$; Fig. 2C). We next evaluated iNOS/β-tubulin ratios with MSK scores and combined these data with our previous patient cohort (24). We confirmed and strengthened our original observations, which showed a significant correlation between iNOS protein expression and the severity of HAD as measured by MSK scores ($\rho = 0.618$, $p \leq 0.001$; Fig. 2D).

The relative level or extent of macrophage or microglial activation was assessed by Western blot analysis of HAM56, a marker that is widely

Table 2. Demographic and clinical characteristics of dementia severity groups[a]

Data	MSK 0 (n = 13)	MSK 1–2 (n = 11)	MSK 3–4 (n = 6)
Age (years)	46.33 ± 7.11	41.73 ± 2.28	43.60 ± 4.09
Survival (months)	n/a	10.11 ± 7.50	7.32 ± 4.18
CD4 count	80.25 ± 38.14	40.60 ± 15.44	44.40 ± 29.04
PMI (hr)	13.54 ± 1.31	15.09 ± 3.15	42.83 ± 32.42
Progression	ND	NP & MP	RP

[a] Total cohort = 30 patients. None of the variables are statistically different at $p \leq 0.05$.

MP, moderate progression; MSK, Memorial Sloan-Kettering score; ND, no dementia; PMI, postmortem interval; RP, rapid progression; NP, non-progression.

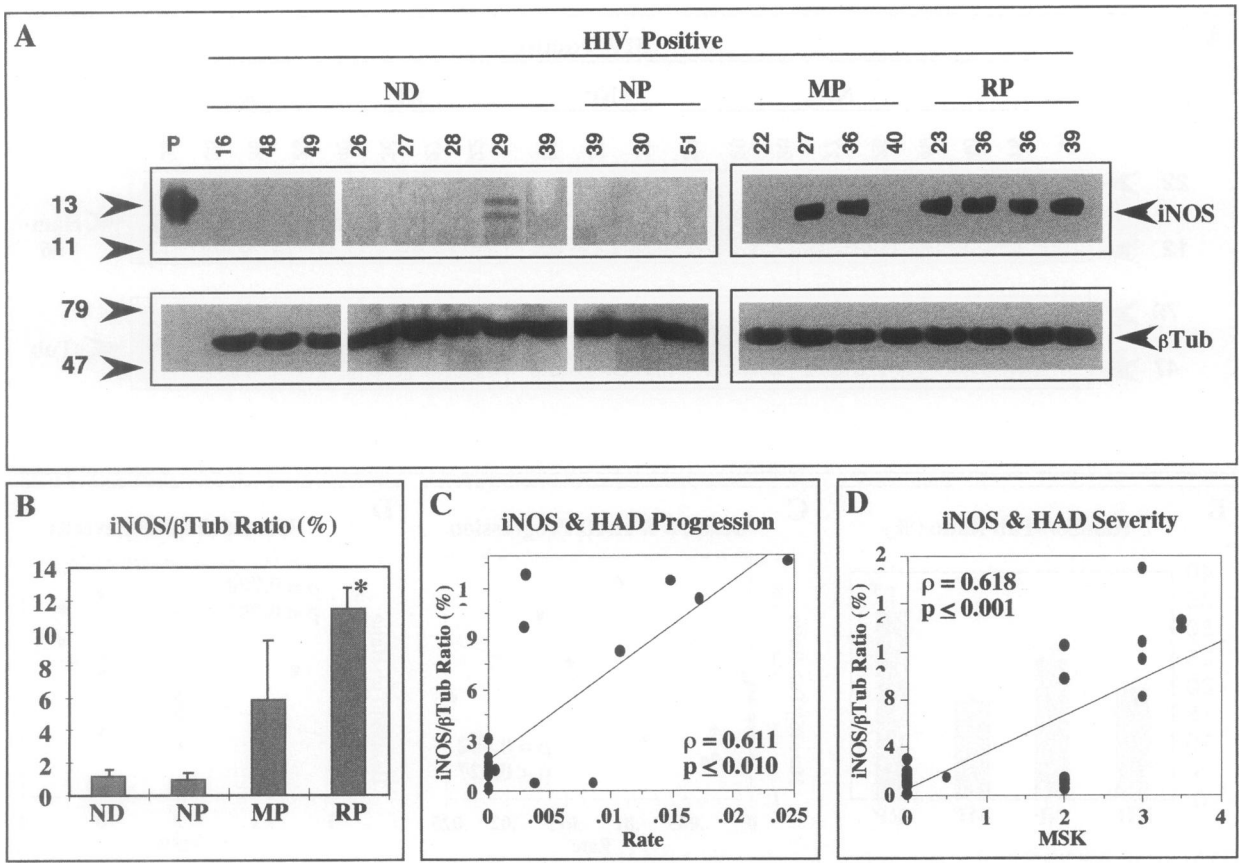

Fig. 2. Expression of iNOS protein coincides with rapid progression of HAD. (A) iNOS and β-tubulin (βTub) protein immunoblots of postmortem cortical tissue from HIV-1-infected patients with no dementia (ND), non-progression (NP), moderate progression (MP), and rapid progression (RP) of HAD. These results were replicated three times with similar results. Positive controls (P) were obtained from lipopolysaccharide-stimulated rodent glial cultures. ND and NP samples were from separate immunoblots (white space between blots) and aligned for comparison. (B) Mean levels of iNOS protein relative to that of β-tubulin from HIV-1-infected patients with ND, NP, MP, and RP. Representative blots are shown in (A). The levels of iNOS were analyzed for significance by means of the Kruskal-Wallis test for multiple groups ($p \leq 0.05$). *Fisher's least-significance difference posthoc test indicated highly significant differences for iNOS (RP to ND, $p \leq 0.0001$; RP to NP, $p \leq 0.0003$; RP to MP, $p \leq 0.0229$). Data are means ± SEM. (C) The Spearman rank correlation test was used to compare iNOS/β-tubulin ratios to progression of HAD and yielded $\rho = 0.611$, $p \leq 0.010$. (D) The Spearman rank correlation test was used to compare iNOS/β-tubulin ratios to severity of HAD and yielded $\rho = 0.618$, $p \leq 0.001$. Eleven additional cases were used in the statistical analysis (24).

used to assess macrophage activation in postmortem tissue (5). We observed a baseline level of HAM56 expression in all samples and a significant increase in expression in the rapid progressor group (Fig. 3A, B). Correlation analysis yielded a moderate but significant positive correlation between HAM56 protein expression and HAD progression ($\rho = 0.502$, $p \leq 0.027$; Fig. 3C). HAM56/β-tubulin ratios were also compared with MSK scores; this comparison included our previous patient cohort (24). We observed a strong and significant correlation between HAM56 levels and the severity of HAD ($\rho = 0.700$, $p \leq 0.001$; Fig. 3D).

The HIV-1 viral proteins gp41, gp120, and p24 levels were also examined via immunoblotting and compared with rate and severity of progression. As in previous reports (24,41,42), we were unable to detect gp120. Like iNOS and HAM56 protein expression, significantly elevated levels of gp41 protein were found in rapid progressors (Fig. 4A, B). Correlation analysis showed a significant correlation between the progression of HAD and gp41/β-tubulin ratios ($\rho = 0.564$, $p \leq 0.017$; Fig. 4C). In the comparison of MSK scores with gp41/β-tubulin ratios we again combined data from this study with those from our previous patient cohort (24). We con-

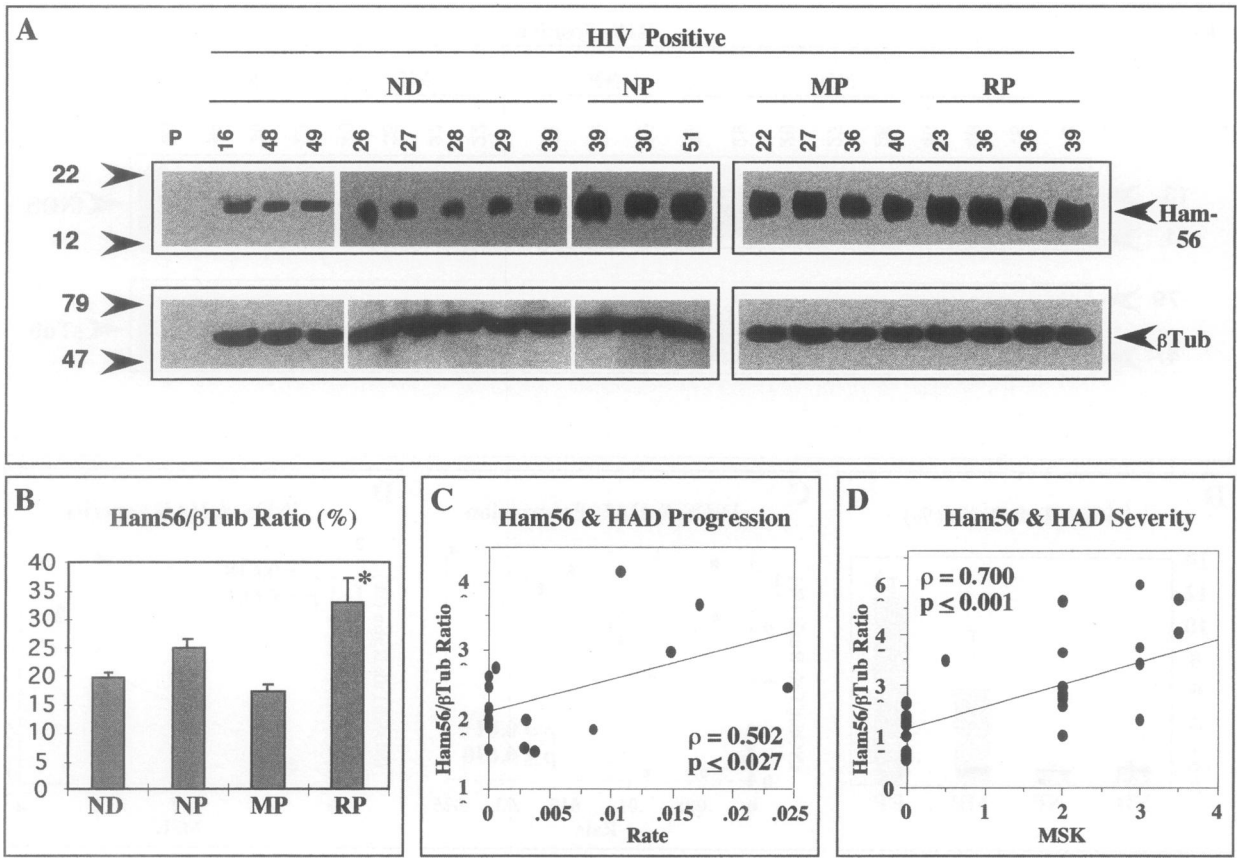

Fig. 3. Expression of HAM56 protein coincides with rapid progression of HAD. (A) HAM56 and β-tubulin (βTub) protein immunoblots of postmortem cortical tissue from HIV-1-infected patients with no dementia (ND), non-progression (NP), moderate progression (MP), and rapid progression (RP) of HAD. These results were replicated three times with similar results. ND and NP samples were from separate immunoblots (white space between blots) and aligned for comparison. P, positive control. (B) Mean levels of HAM56 protein relative to that of β-tubulin from HIV-1-infected patients with ND, NP, MP, and RP. Representative blots are shown in (A). The levels of HAM56 were analyzed for significance by means of the Kruskal-Wallis test for multiple groups ($p \leq 0.006$). *Fisher's least significance difference posthoc test indicated highly significant differences for HAM56 (RP to ND, $p \leq 0.0001$; RP to NP, $p \leq 0.0137$; RP to MP, $p \leq 0.0001$). Data are means ± SEM. (C) The Spearman rank correlation test was used to compare HAM56/β-tubulin ratios to progression of HAD and yielded $\rho = 0.502$, $p \leq 0.027$. (D) The Spearman rank correlation test was used to compare HAM56/β-tubulin ratios to severity of HAD and yielded $\rho = 0.700$, $p \leq 0.001$. Eleven additional cases were used in the statistical analysis (data not shown).

firmed and extended our prior observations that a strong and significant correlation exists between gp41 levels and MSK scores ($\rho = 0.709$, $p \leq 0.001$). In a comparison of p24 levels with rates of progression and MSK scores, p24 levels were not correlated with rate ($\rho = 0.171$, $p \leq 0.467$) or MSK scores ($\rho = 0.284$, $p \leq 0.114$; Fig. 5).

Discussion

In the present study we show that the severity and rate of progression of HAD correlates significantly with levels of the HIV-1 coat protein, gp41, iNOS, and HAM56, a marker of microglial/macrophage activation. Our observations extend and strengthen our previous studies in which we showed that only the severity of HAD correlated with indices of immune system activation (5) and with levels of iNOS and gp41 (24). Other investigators have observed mRNA or protein for iNOS (24,26,43) in CNS tissue from HIV demented individuals. The failure of Bagasra et al. (44) to detect iNOS mRNA by in situ PCR may be due to a lack of prospective analysis and staging of HAD patients, as iNOS protein is only detectable in moderate to severely demented HIV-1-

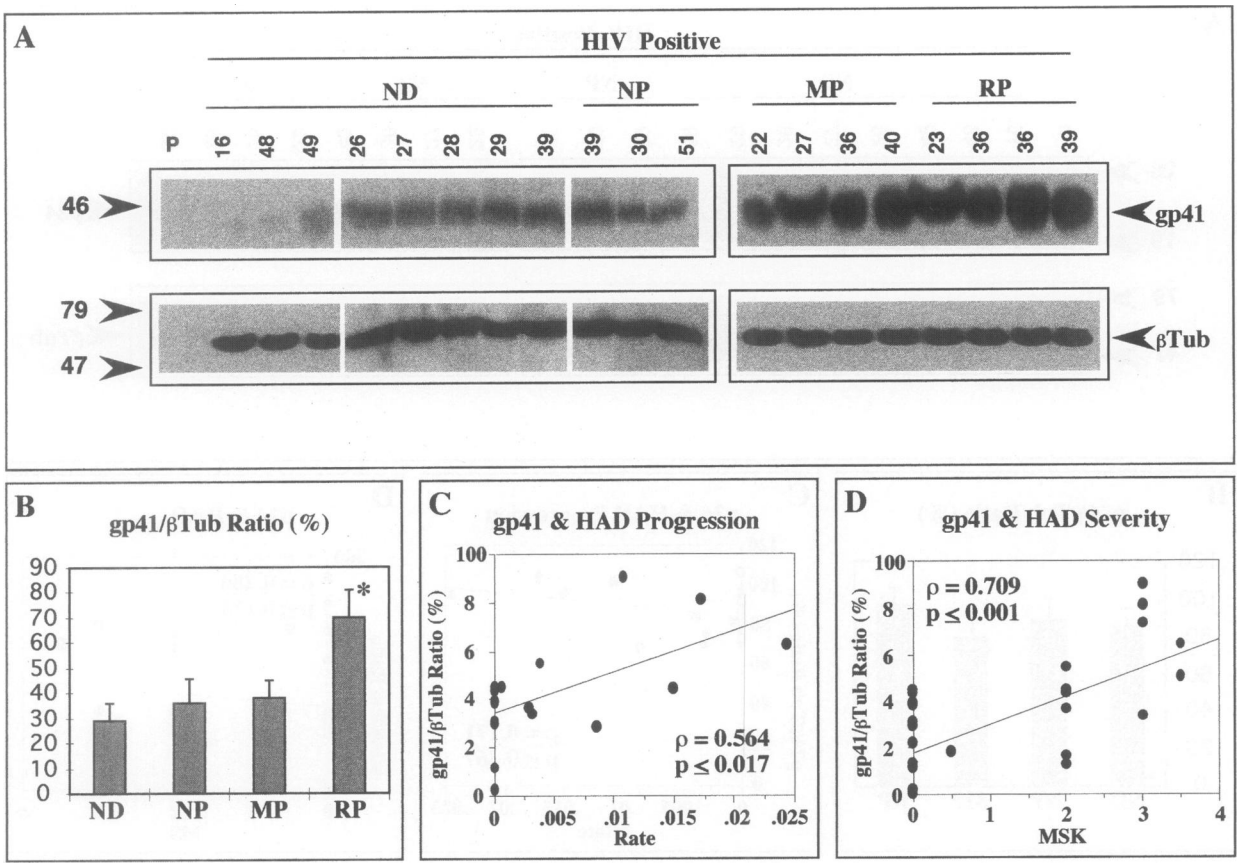

Fig. 4. Expression of HIV-1 gp41 protein coincides with rapid progression of HAD. (A) gp41 and β-tubulin (βTub) protein immunoblots of postmortem cortical tissue from HIV-1-infected patients with no dementia (ND), non-progression (NP), moderate progression (MP), and rapid progression (RP) of HAD. These results were replicated three times with similar results. ND and NP samples were from separate immunoblots (white space between blots) and aligned for comparison. P, positive control. (B) Mean levels of gp41 protein relative to that of β-tubulin from HIV-1-infected patients with ND, NP, MP, and RP. Representative blots are shown in (A). The levels of gp41 were analyzed for significance by means of the Kruskal-Wallis test for multiple groups ($p \leq 0.05$). *Fisher's least-significance difference posthoc test indicated highly significant differences for iNOS (RP to ND, $p \leq 0.0020$; RP to NP, $p \leq 0.0082$; RP to MP, $p \leq 0.0130$). Data are means ± SEM. (C) The Spearman rank correlation test was used to compare gp41/β-tubulin ratios to progression of HAD and yielded $\rho = 0.564$, $p \leq 0.017$. (D) The Spearman rank correlation test was used to compare iNOS/β-tubulin ratios to severity of HAD and yielded $\rho = 0.709$, $p \leq 0.001$. Eleven additional cases were used in the statistical analysis (24).

infected patients (24). There appears to be a threshold effect in which high levels of gp41, iNOS, and HAM56 are particularly associated with severe (MSK 3 to 4) and rapidly progressive HAD. Thus, iNOS, gp41, and macrophage/microglial activation are the first neuropathogical variables identified that correlate with the rate of progression of HAD. We have previously reported no statistically significant difference among these groups in the amount of brain atrophy, white matter hyperintensity, microglial nodules, multinucleated giant cells, perivascular cuffs, diffuse myelin pallor, multifocal white matter lesions, infarction, vacuolar myelopathy, cytomegalovirus (CMV) inclusions, or gliosis (36). Although gp41 appears to play a prominent role in the rate and severity of progression of HAD, it is unlikely to be the sole or primary insult in HAD as a number of host and viral factors have been shown to modulate or contribute to HAD. These other factors may be particularly important in less severe cases of HAD.

Previous studies have shown a wide variability in the rates of progression of HAD among HIV-infected individuals (2,20,36,45). We have previously identified unique groups within this spectrum which we designated non-, moderate, and rapid progressors (36). Non- and moderate

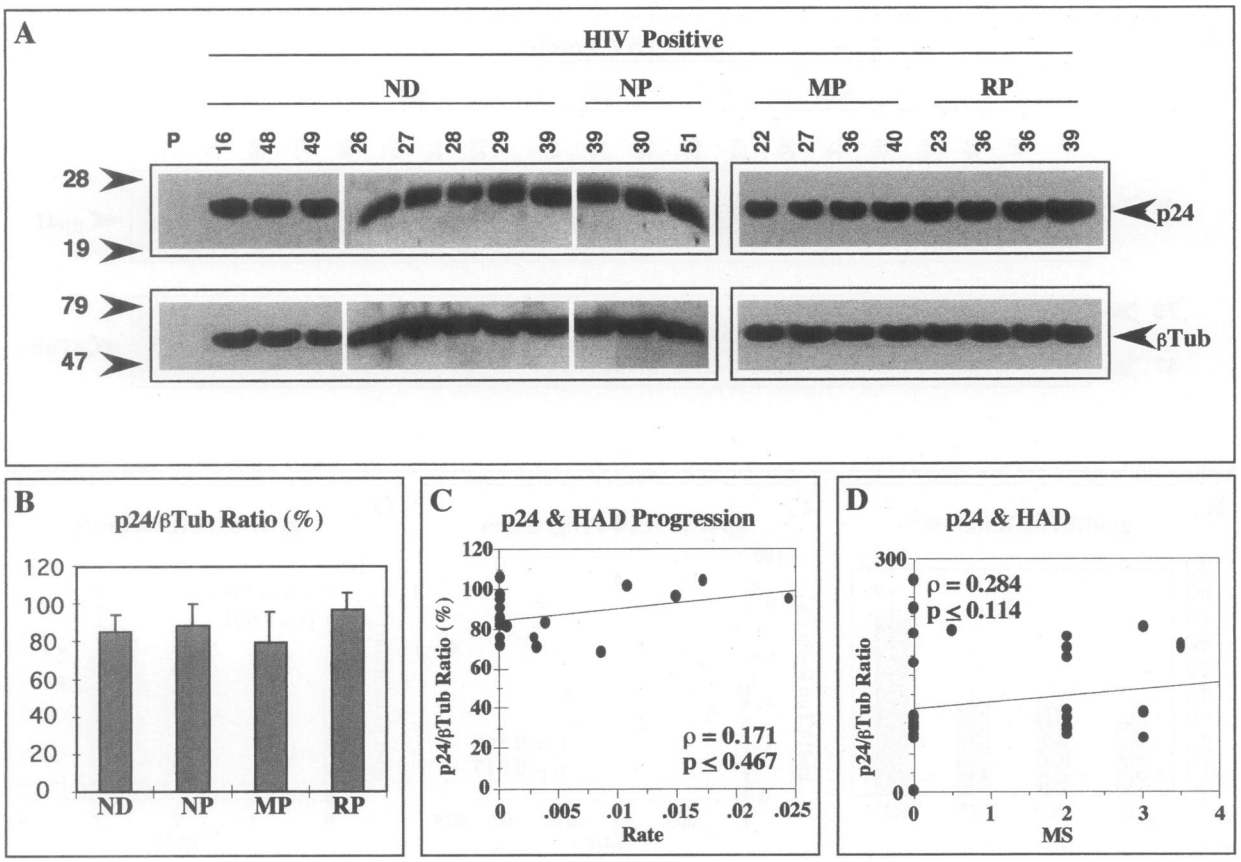

Fig. 5. Expression of HIV-1 p24 viral protein does not coincide with rapid progression of HAD. (A) p24 and β-tubulin (βTub) protein immunoblots of postmortem cortical tissue from HIV-1-infected patients with no dementia (ND), non-progression (NP), moderate progression (MP), and rapid progression (RP) of HAD. These results were replicated three times with similar results. Positive control (P) was used for iNOS protein. ND and NP samples were from separate immunoblots (white space between blots) and aligned for comparison. (B) Mean levels of p24 protein were relative to that of β-tubulin from HIV-1-infected patients with ND, NP, MP, and RP. Representative blots are shown in (A). The levels of p24 were analyzed for significance by the Kruskal-Wallis test for multiple groups ($p \leq 0.22$). Fisher's least-significance difference posthoc test did not indicate significant differences for p24 (RP to ND, $p \leq 0.1311$; RP to NP, $p \leq 0.3231$; RP to MP, $p \leq 0.0513$). Data are means ± SEM. (C) The Spearman rank correlation test was used to compare p24/β-tubulin ratios to progression of HAD and yielded $\rho = 0.171$, $p \leq 0.467$. (D) The Spearman rank correlation test was used to compare p24/β-tubulin ratios to severity of HAD and yielded $\rho = 0.284$, $p \leq 0.114$. Eleven additional cases were used in the statistical analysis (24).

progressors have a very slow neurological progression over a course of months to years, whereas the rapid progressors show rapid increases in neurological severity over weeks to months (36). One limitation in our data is that the rate of neurological progression was determined from only two MSK scores, assuming a linear change. Such an assumption easily overlooks a stepwise or multiphasic change, e.g., an initially slow change followed by a rapid change. Prior studies have indicated that there is no difference in progression to AIDS according to sex, race, or risk factor for HIV infection (30,45), whereas others have shown a difference according to race, risk factor, CD4 count, and survival to death (46). Since we wanted to examine potential differences in protein markers with rate of progression, we controlled for these factors by choosing groups with no difference in CD4 count and survival (Table 1). Additionally, we attempted to control for protein degradation by choosing cases with similar postmortem intervals from tissue procurement to freezing, and simultaneously preparing all the samples in an identical fashion.

The striking and statistically significant eleva-

tion of gp41/β-tubulin ratios in severe and rapidly progressing HAD and the failure to detect gp120 are puzzling. Other investigators have also failed to detect gp120 in the brains of HAD patients (41,42). Theories on this failure suggest that gp120 is rapidly degraded by extracellular proteases, thus preventing its detection in brain. Alternatively, it has been suggested that antibodies used to detect gp120 do not recognize gp120 in the brain because of the specificity of the antibodies used. However, these antibodies readily detect gp120 in the periphery of HIV-infected patients by both immunohistochemical and Western blot approaches (47–49). Recently, SIV-infected macaques with neurologic disease were also found to have levels of gp41 that correlated with the extent of disease, whereas viral load failed to show any correlation (J. Clements and M. C. Zink, personal communication). Thus, it is becoming clear that there are complexities in the regulation and expression of HIV proteins, particularly gp41, that may play important roles in the pathogenesis of HAD. This dissociation between gp41 expression patterns and p24 expression patterns is puzzling. It is not likely due to cross-reactivity of the p24 or gp41 antibodies with a human protein, as we observed only a single band on immunoblot that is present only in HIV-infected patients (see Patients and Methods, ref. 24). Changes in protein expression patterns are due to either increased translation or decreased degradation. In the SIV model a single point mutation in the gp41 region of env results in a virus that causes neurologic disease and increased gp41 expression which does not directly correlate with viral message (50). It is unlikely that a single point mutation in gp41 would confer increased viral translation, although it is likely that a point mutation would alter the rate of protein degradation. Little is known about the kinetics of HIV protein expression in the CNS. In COS cell lines env expression kinetics are regulated by rev and tat and by the efficiency of proteolytic cleavage of gp160 (51), while expression of Pr55gag inhibits internalization and degradation of gp41 (52). In the CNS where viral replication and maturation is slowed, gp41 will not be lost due to the production of viral particles, and if degradation is inhibited by expression of Pr55gag, then it is feasible that gp41 would accumulate, perhaps in relation to soluble HIV proteins that would be exposed to proteolytic processes.

In vitro studies in both rodent and human glial cultures indicate that full-length and appropriately post-translationally modified gp41 expressed on cellular membranes by vesicular stomatitis virus (VSV) is a potent inducer of TNF-α, IL-1β, and iNOS (53,54). Interestingly, recombinant bacterially expressed gp41 containing the extracellular domain obtains appropriate tertiary structure (55,56) and behaves in an identical manner to full-length gp41 in inducing TNF-α, IL-1β, and iNOS (24,54,57). Thus, the elevated levels of gp41 in severe and rapidly progressing HAD may act as an activator of the immune system and elicit a sustained immunologic reaction that triggers pathogenic processes, ultimately leading to severe and rapid progression of HAD. Consistent with this notion is our observation that subnanomolar physiologically relevant concentrations of recombinant gp41 is neurotoxic to rodent cultures and kills neurons in an NO-dependent fashion from induction of iNOS (24,57). Recent studies indicate that recombinant gp41 is also toxic to human neuronal cultures in an iNOS/NO–dependent fashion (L. Pulliam, personal communication). We propose that, like nonsecreted membrane proteins that induce signaling processes through cell-to-cell interactions, the extracellular portion of gp41 expressed in infected cells interacts with adjacent cells to cause macrophage/microglial activation and induction of iNOS. This is particularly important in the latter stages of HAD. Strategies aimed at reducing levels of gp41 or interfering with signaling cascades induced by gp41 may have therapeutic benefit.

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